I hereby certify that this paper is being facsimile transmitted to the United States Patent and Trademark Office on the date shown below.

Christi Butner
Christi Butner
Date of Signature: April 18, 2005

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Gunzburg and Saller — Group Art Unit: 1631

Serial No.: 08/808,827 — Examiner: Brusca, John S.

Filed: February 28, 1997 — Docket No.: 1406/194

Confirmation No.: 6837

For: NON SELF-INACTIVATING, EXPRESSION TARGETED RETROVIRAL VECTORS

* *

DECLARATION OF CHRISTINE LEIB-MOESCH
PURSUANT TO 37 C.F.R. §§1.132

Commissioner of Patents
Washington, D.C. 20231

Sir:

1. My name is Christine Leib-Moesch, and I am currently research project leader at the GSF-Forschungszentrum fuer Umwelt und Gesundheit GmbH, assignee for the subject U.S. Patent Application Serial No. 08/808,827.

2. A true and accurate copy of my *curriculum vitae*, which evidences my expertise and credentials, is attached herewith and labeled **Exhibit A**.

3. I have had an opportunity to review pending claims 1, 5, 7, 9-26, 28, 29, and 31-78 in the subject above captioned U.S. Patent Application Serial No. 08/808,827.

4. I have also reviewed the following documents: the Final Official Action issued October 24, 2004 on the above captioned U.S. Patent Application Serial No. 08/808,827 by the U.S. Patent and Trademark Office; Couture *et al.* (5 *Human Gene*

Therapy 667-677, 1994; hereinafter "Couture"); and Faustinella *et al.* (5 *Human Gene Therapy* 307-312, 1994; hereinafter "Faustinella") cited in the Official Action.

5. Couture describes retroviral vectors characterized by substitutions of portions of the 3' U3 regions with corresponding regions of 5 related murine retroviruses in order to create retroviral vectors with complete, chimeric 3' LTRs that display different tissue tropisms based on the presence of regulatory elements present within the U3 regions of the chimeric 3' LTRs. The replacement strategies disclosed in Couture produce complete chimeric LTRs "based on the substitution of the MoMLV U3 region with the U3 region from the murine retroviral isolates SL3-3, AKV, Xeno, HaMSV, and MPSV" (Couture at page 669). This was accomplished by employing conserved restriction sites present in the 3' LTRs of these retroviruses. As such, the vectors disclosed by Couture were specifically designed to have complete U3 regions.

6. There is no disclosure in Couture of any retroviral vector in which the U3 region of the 3' LTR contains a deletion. Rather, Couture teaches producing complete, although chimeric, 3' LTRs by "swapping" corresponding regions of the 3' U3 sequences of five related retroviruses into the vector.

7. Faustinella describes a MoMLV-based vector characterized by a partial deletion of the 3' U3 region into which a polylinker has been inserted. The strategy described in Faustinella is used to create self-inactivating vectors by deletion of the promoter/enhancer sequences present within the 3' LTR. The polylinkers were then added to facilitate the cloning of a promoter operatively linked to a coding sequence. Given that the purpose of the Faustinella strategy was to create self-inactivating vectors (SIN vectors), if the 3' LTR polylinker is to be used as a cloning site for a promoter, that promoter must be operatively linked to a gene of interest, because only when the inserted promoter is operatively linked to a gene of interest is a self-inactivating vector produced. Thus, Faustinella discloses two main strategies: (a) the deletion of regulatory sequences from the 3' U3 to create a SIN vector, which requires that the 3' U3 remain without regulatory sequences; and (b) the use of a polylinker to clone promoter/coding sequence pairs into the 3' U3 deletion, because if

a promoter is cloned into the polylinker, then the SIN character of the vector is destroyed unless a coding sequence is operatively linked to this promoter.

8. There is no disclosure in Faustinella of any retroviral vector in which the U3 region of the 3' LTR contains a deletion and a heterologous promoter without there also being present a coding sequence operatively linked to the heterologous promoter in the 3' U3.

9. The claimed subject matter of the subject U.S. Patent Application Serial No. 08/808,827 relates to retroviral vectors that are characterized by the complete or partial deletion of the 3' U3 region and the replacement of part or all of the 3' U3 region with heterologous promoters and/or regulatory elements, which are then used to regulate expression of coding sequences that are present within the body of the vectors (*i.e.* are not operatively linked to the promoters and/or regulatory sequences in the vectors). Upon infection of a target cell, these heterologous promoters and/or regulatory elements become operatively linked to one or more coding sequences present within the body of the vector. As a result, the coding sequences present within the body of the vector, which can encode therapeutic polypeptides such as antiviral genes, antitumor genes, cytokine genes and combinations thereof, are expressed in those tissues and cell types in which the heterologous promoters and/or regulatory elements are active.

10. The claimed subject matter of the subject U.S. Patent Application Serial No. 08/808,827 provides several safety benefits. First, since the regulatory elements normally found within the 3' U3 sequences of the retroviral vector are removed, the therapeutic gene is not expressed except in those tissues in which the heterologous promoter and/or regulatory elements are active. Thus, particularly in those embodiments where the coding sequences encode a suicide gene, incorporation of the retroviral vector into non-target cells should not result in adverse side effects because the coding sequences are not expressed. Second, the use of heterologous promoters and/or regulatory elements reduces the likelihood that recombination between the vector and sequences present within either the cell lines used to create the vector or endogenous sequences in the host will result in the production of replication competent vectors. This is particularly important for the therapeutic use of

retroviral vectors, because while it is believed that most researchers assume that the probability of a replication defective vector integrating into the genome of the target cell and insertionally activating an oncogene near the insertion site is small, if a replication competent retrovirus were produced, the expansion of the population of replication competent viruses would be expected to increase this probability exponentially. Given that the instantly claimed retroviral vectors comprise partially or completely deleted 3' U3 regions and employ heterologous promoters therein, the probability of generating a replication competent virus from the instantly claimed retroviral vectors is believed to be much lower than when using the Couture vectors, which employ complete 3' LTRs that have 3' U3 sequences that are highly homologous to wild-type retroviruses.

11. The deletion of 3' U3 sequences and insertion of heterologous promoters into the 3' U3 is not suggested by the combination of Couture and Faustinella as contended by the United States Patent and Trademark Office. While Couture appears to suggest that replacement of 3' U3 sequences with the corresponding sequences from related retroviruses to restore a complete 3' U3 region might result in expression of coding sequences present within the body of the vector, Couture also states a correlation between expression levels and the degree of homology between the sequences that were exchanged. According to Figure 2 of Couture, the homology between the Mo-MuLV U3 that was removed and the U3 regions that were inserted was 99% for HaMSV, 98% for MPSV, 76% for AKV, 74% for SL3-3, and 64% for Xeno. Table 3 of Couture indicates that the ability of the chimeric LTRs to direct expression of the coding sequence correlated with the degree of homology to Mo-MuLV in each cell line tested. Thus, Couture strongly suggests that as the degree of homology between the Mo-MuLV U3 region that is removed and the promoter that is inserted decreases, the ability of the promoter to regulate expression of a gene present within the body of the vector also decreases. Given that these vectors are contemplated for therapeutic use in subjects, the expression level that can be generated by the recombinant vector is of importance. Thus, no scientific basis is believed to be found in Couture and Faustinella to motivate the use of a polylinker to construct the vectors, as disclosed by Faustinella, since the

since the presence of a polylinker would reduce the homology levels in reconstructing the complete 3' U3 region as disclosed by Couture, which would be expected to reduce expression levels based on the above-mentioned observation in Couture.

12. It is also believed that Couture teaches against the use of cellular promoters because the sequences of such promoters would be expected to have even less homology to Mo-MuLV than did the Xeno sequences disclosed in Couture, and thus would be expected to result in low and likely unsatisfactory expression levels. Thus, no scientific basis is believed to be found in Couture and/or in Faustinella to motivate the use of promoter and/or regulatory sequences from cellular genes, and correspondingly, the use of a polylinker to construct the vectors. Thus, it is believed that there would be no motivation to look to Faustinella having considered the teachings of Couture to construct a retroviral vector.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Respectfully submitted,

Christine Leib-Moesch

14 April 2005

Date

Attachment: Exhibit A